

in an atmosphere of CO<sub>2</sub>:O<sub>2</sub>:N<sub>2</sub> (8:8:84). Supernat was removed and the cells were treated with 0.2% KCL for 5 min. and 0.1% KCL for 25 min. The KCL was removed and the cells were harvested by scraping. The harvested cells were passed through a 22 gauge needle to break down the cell structure. The cell lysate was subjected to low speed centrifugation for 10 min. and the semi-purified organisms remaining in the supernatant were harvested by high speed centrifugation. Antigen was pooled from 25 flasks and a portion of the antigen was subjected to a french press treatment for the production of soluble antigen. The Remainder was aliquoted and stored at -70°C. This soluble antigen was formulated into a vaccine according to the following procedure. Vaccine antigen was formulated with TITERMAX®, a block copolymer/metabolizable oil adjuvant, or Freunds Incomplete adjuvant at a concentration of 500ug of antigen/dose. With the TITERMAX® adjuvant, 0.5mL was mixed with 0.5mL of antigen to produce a 1.0mL dose containing 500ug of antigen. With the Freunds Incomplete adjuvant, 2.0mL of adjuvant was mixed with 2.0mL of antigen such that the total dose also contained 500ug.

B' In order to determine whether the antigen produced could protect pigs from a homologous challenge or from exposure to heterologous isolates or strains, ten 4-week-old pigs were vaccinated and later challenged. Ten control pigs received equal doses of a mock vaccine which contained only the tissue culture medium Minimal Essential Medium (MEM)) and adjuvant (without antigen). The vaccine used for the first vaccination contained TITERMAX® adjuvant while the vaccine used for the second vaccination contained Freunds Incomplete adjuvant. Serum samples were taken prior to vaccination (prebleed), at day of booster (Day 14) and at the day of challenge (Day 35) to demonstrate the production of an immune response post vaccination. Serum was tested for antibody to *L. intracellularis* via an ELISA wherein the wells in a 96-well plate were coated with *L. intracellularis* antigen (purified from pig gut epithelial cells) of a clinical isolate which was from a different source than the isolate used to produce the vaccine. Therefore, presence of an

**In the Claims (Clean Sheet)**

Please amend claims 3-5, 27-29 and 31 as follows:

- B<sup>2</sup>
3. (amended) The immunogenic composition according to Claim 27 further comprising an inactivating agent.
  4. (amended) The immunogenic composition according to Claim 3, wherein the inactivating agent is selected from the group consisting of formalin, beta-propiolactone, heat, binary ethylenimine, detergents, and freeze/thaw.
  5. (amended) The immunogenic composition according to Claim 3, wherein the adjuvant is selected from the group consisting of polymers, oil in water, water-in-oil-in-water, lipids, aluminum hydroxide, aluminum phosphate, aluminum sulfate, immunomodulators and combinations thereof.
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- B<sup>3</sup>
27. (amended) An immunogenic composition comprising inactivated *L. intracellularis* antigen, wherein the *L. intracellularis* has all the immunogenic characteristics of ATCC deposit No. 55370, and an adjuvant, and wherein the immunogenic composition induces an immune response resulting in the production of protective antibodies in a swine to which it has been administered that react with at least one antigen selected from the group consisting of *L. intracellularis* ATCC deposit No. 55370 antigens having the molecular weights of 21 kDa, 31 dDa, 41

kDa, 43 kDa, 44 kDa, 60 kDa, 71 kDa, and 115 kDa.

28. (amended) The immunogenic composition according to claim 27, wherein the *L.intracellularis* antigen comprises lysate of whole tissue culture grown *L.intracellularis*.

29. (amended) The immunogenic composition according to Claim 27, wherein the *L. intracellularis* antigen comprises an inactivated tissue culture of *L.intracellularis*.

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31. (amended) A method for protecting swine from disease caused by *L. intracellularis* comprising, administering to said swine an effective amount of the immunogenic composition according to claim 27.

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